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Proteus bacteria species from hospital sewage and Mfoundi River in Yaounde (Cameroon, Central Africa): Comparison of the diversity, abundance and susceptibility against some β-lactams, Quinolones and Aminoglycosides antibiotics

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Abstract
Species of the genus *Proteus* are the cause of several infections and represent one of the microorganisms commonly involved in hospital infections. These infections are sometime difficult to treat because the antibiotic resistance phenomenon, which represents one of the greatest health challenges today. This study aimed at comparing the diversity, abundance and antimicrobial susceptibility of *Proteus* species isolated from hospital wastewater and Mfoundi River in Yaounde (Cameroon, Central Africa). The physicochemical parameters were measured using appropriate techniques while bacteria were isolated using standard methods and identified using the API 20E systems. The antibiogram tests were carried out using the Müller-Hinton antibiotic disc diffusion method. Antibiotics used belonged to the β-lactam, Quinolones and Aminoglycoside groups. The results obtained show that these waters are slightly alkaline (pH>7.5) and contain dissolved ions (electrical conductivity>600µS/cm; TDS>120mg/L). These waters host various *Proteus* species such as *P. mirabilis*, *P. penneri* and *P. vulgaris*, which were mostly represented in hospital wastewater. The prevalence of antimicrobial resistance varied with respect to the *Proteus* species and antibiotic groups. All *Proteus* strains were resistant to β-lactams and Quinolones. Besides, 41.8% of strains of isolated species were resistant to Gentamycin and 87.5% were sensitive to Amikacin. Most of the bacteria strains isolated in wastewater and surface water were resistant to all the antibiotics tested. Many bacterial strains tested were multi-resistant (82.76%). This multidrug resistance was more expressed in *P. mirabilis* and *P. vulgaris* species isolated from wastewater and upstream river. This represents a health risk for humans and the aquatic environment.

Keywords: Abiotic factor, antibiotic susceptibility, cells abundance, *Proteus* bacteria species, river, waste water

Introduction
Species of the genus *Proteus* are members of the *Morganellaceae* family but, according to Dai *et al*. [1], this genus belongs to *Enterobacteriaceae* family. All of the species are motile, lactose-negative, urease-producing, Gram-negative, rod-shaped bacteria capable of differentiating from typical enterobacterial bacilli into highly elongated rods covered with thousands of flagella, producing swarming colonies [2, 3, 4]. They are widespread in nature and are isolated in surface water, wastewater, soil, on vegetables and, in the putrefactive flora of animal organic matter. They vegetate as saprotrophes on the skin and mucous membranes, and are the usual hosts of the digestive tract of humans and animals [5].
The genus *Proteus* includes several species, namely: *Proteus alimentorum*, *Proteus cibarius*, *Proteus columbae*, *Proteus faecis*, *Proteus hauseri*, *Proteus mirabilis*, *Proteus penneri*, *Proteus terrae*, and *Proteus vulgaris* [1]. Although there are many species of the genus *Proteus*, the majority of clinical strains isolated are *Proteus mirabilis* and *Proteus vulgaris* [2]. The abundance and diversity of species of the genus *Proteus* varies according to their

environment.

According to Bawa *et al*. [6], botanical gardens harbour a high diversity of *Proteus* species (*Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, *Proteus spp.*) with low bacterial abundance; whereas in urine, *Proteus* species are diverse and highly abundant [7]. The same applies to wastewater and surface water, where there is a high diversity and abundance of *Proteus*.

Species of the genus *Proteus* are often involved in urinary tract infections and come third after *Escherichia coli* and *Klebsiella spp*. [8, 9]. They also cause wound infections, superinfection of various respiratory tract tumours [5]. Indeed, these infections constitute a real public health problem and are second only to respiratory infections [10].

The bacterial epidemiology of urinary tract, wound and lung infections has changed significantly over the last 20 years [11]. The bacteria involved are increasingly varied and, above all, have become more resistant to antibiotics [12, 13]. No bacterial species, among those found in human pathology, and no antibiotic, even among the most recent, escapes the phenomenon of resistance today, especially in urinary infectious pathology [14, 15, 16], which in some cases results in therapeutic failure. These cases of therapeutic failure are due to the fact that bacteria develop various resistant mechanisms including the secretion of enzymes such as β-lactamases in order to survive in the environment.

Previous studies have confirmed that β-lactam resistance is currently emerging in bacteria of the genus *Proteus* [17] as they secrete β-lactamases to inhibit the action of antibiotics. Esmail *et al*. [18] pointed out in their work that *Proteus* isolates are 100% resistant to Sulphamethoxazol + Trimethoprim, Cefotaxin, Amikacin and Ceftazidim and 75% to Tobramycin, Amoxicillin + Clavulanic acid, Nitrofurans, Cefalotin and Amoxicillin. However, this genus is full susceptibility to Ceftriaxon, Nalidixic acid, Ciprofloxacin. Abbott *et al*. [19] have shown that *Proteus* species are generally susceptible to Cephalosporins, Aminoglycosides and broad spectrum Imipenem. These antibiotics to which the bacteria are resistant are those most commonly used in therapy.

Many studies of antibiotic resistance in *Proteus* species have been conducted on clinical cases. Few studies have focused on *Proteus* species isolated from aquatic environments. Yet these environments contribute to the spread of antibiotic resistance in bacteria. According to Souna [11], regular monitoring of the susceptibility of the predominant bacterial species to the various antibiotics in common use is essential. The present work aims to make a comparative study of the diversity and abundance of bacterial species of the genus *Proteus* isolated from hospital wastewater and the Mfoundi River of the city of Yaounde (Cameroon - Central Africa) and their susceptibility to some antibiotics belonging to the β-lactams, Quinolones and Aminoglycosides families.

2. Material and methods

2.1. Study area

This study was carried out in Yaounde, the capital of Cameroon, located 300 km from the Atlantic coast, between 3°5' North latitude and 11°31' East longitude [20]. The climate is equatorial, characterised by the alternation of two dry seasons and two rainy seasons: a long dry season from December to mid-March, a short rainy season from mid-March to June, a short dry season from July to August and a long rainy season from September to November. The annual average temperature is 23.5 °C, varying between 16 and 31 °C depending on the season, and 1650 mm of water per year. The city's hydrographic network is very dense and essentially composed of the Mfoundi River and its tributaries. Some districts and hospitals in Yaounde are equipped with wastewater treatment plants.

2.2. Sampling sites and water sampling

Two kinds of sampling sites were chosen for this study: wastewater of the University Teaching Hospital (UTH) and surface water (Mfoundi River). The wastewater from the laundry and the surgery room of the UTH was coded Sw. The surface water included 3 sampling points: upstream, landing, and downstream coded Ws1, Ws2 and Ws3 respectively. A total of 4 sampling sites were chosen. They are presented in Figure 1 and their characteristics and geographic coordinates are indicated in Table 1.

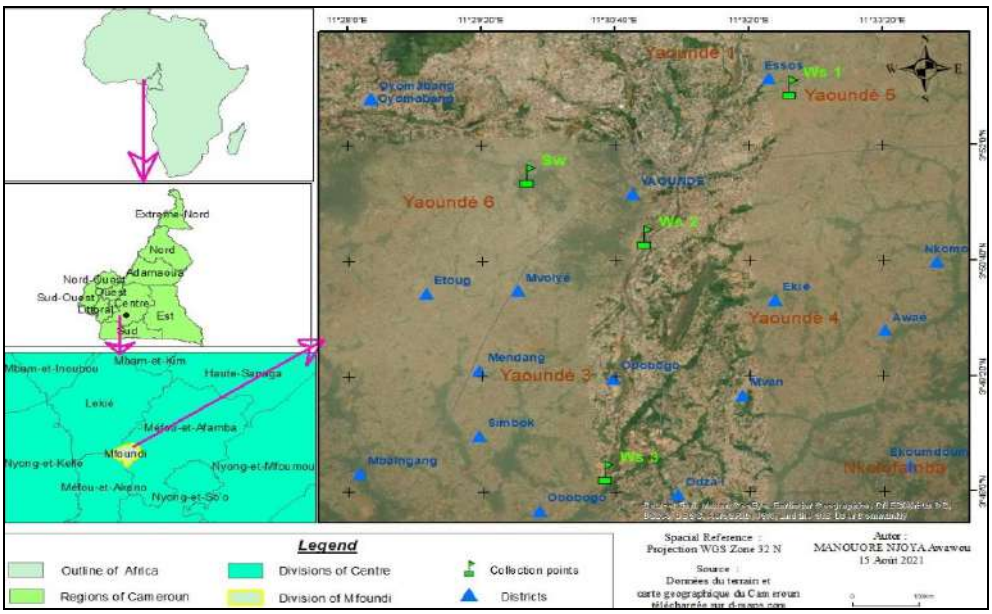


Fig 1: Geographical location of the study area and representation of the sampling points (Source: https//d-maps.com/pays.php?num_pay=16&lang=fr/consulted 25th August 2021)

Table 1: Characteristics of sampling points

Hydro-systems	Sampling points	Geographical coordinates		Description
		Latitude (N)	Longitude (E)	
Hospital wastewater (Sw)	Sw	3°86'09,47''	11°496'49,6''	Collector receiving wastewater from the laundry and the surgery room of the University Teaching Hospital
Surface water (Ws)	Ws1	3°87'77,22''	11°54'01,3''	Upstream of the Mfoundi river, close to houses and where domestic waste is dumped
	Ws2	3°84'90,11''	11°51'59,68''	Landing on the Mfoundi river, receiving domestic waste
	Ws3	3°80'36,78''	11°50'93,25''	Downstream of the Mfoundi river, near a brewery company

Water sampling was done according to Rodier *et al*. [21]. For the bacteriological analyses, around 300 mL of water were collected in 500 mL sterile glass bottles. The samples were then brought back to the laboratory in a refrigerated chamber (4 °C) for analysis. Sampling was done monthly during 12 months from September 2020 to August 2021.

2.3. Physicochemical analysis
Physicochemical parameters were analyzed according to Rodier *et al*. [21] and APHA [22]. The parameters considered were: temperature, pH, electrical conductivity, and Total Dissolved Solids. They were measured in the field using a HANNA/HI 9829 multimeter.

2.4. Bacteriological analysis
2.4.1. Isolation of Heterotrophic Aerobe Bacteria (HAB) and *Proteus* species
Heterotrophic Aerobe Bacteria (HAB) were isolated in plate count agar medium at 25°C±2°C during 5 days incubation. The isolation and counting of *Proteus* species were performed on MacConkey Agar culture medium using plate count technic method. A volume of 0.1 mL of raw/diluated water of each sample was placed on the agar surface in Petri dishes. Incubation was done at 37°C for 24 hours for *Proteus* [21, 23]. All the analyses were done in triplicate.

2.4.2. Macroscopic examination and identification of *Proteus* species
After the incubation period, colonies were counted in different Petri dishes based on their characteristics. *Proteus* colonies have colorless to beige, with filamentous forms of different sizes [23, 24, 25]. After counting the colony forming units based on the cultural characteristics of the bacteria, the cells of colony with different characteristics were recultured on standard (non-selective) agar, and then in the sloping test tubes. After Gram coloration, biochemical tests were performed using the API 20E systems (Biomerieux) [26].

2.4.3. Antibiogram tests
Antimicrobial susceptibility testing was done using disc diffusion method according to the recommendations of the "Antibiogram Committee of the French Microbiology Society" (AC-FMS) [27]. Antibiotic molecules were chosen according to the AC-FMS recommandations for *Proteus* antimicrobial susceptibility testing, but also to their availability in the laboratory.

A total of 17 antibiotic belonging to three main families were used. β-lactams were the most represented family with Amoxicillin; Amoxicillin + Clavulanic acid; Imipenem; Meropenem; Ticarcillin; Piperacillin; Piperacillin + Tazobactam; Ceftriaxon; Cefepim; Cefuroxim; Cefoxitin and Ceftazidim. The family of Quinolones was represented by Ciprofloxacin; Norfloxacin and Ofloxacin; and the family of Aminoglycosides included Amikacin and Gentamycin. Inhibition diameters were measured using the caliper and the results were scored as either resistant, sensitive or intermediate according to the CA-SFM recommendations [28, 29].

2.5. Data analysis
The abundances of isolated bacteria were expressed as CFU/100mL. The values of physicochemical parameters and bacterial abundances were illustrated by histograms plotted using Excel 2016 software. The Kruskal-Wallis and Mann-Whitney tests were carried out to compare inhibition diameters of antibiotic tested between the 4 sampling sites. The Spearman correlation test was achieved to assess the possible relations between abiotic parameters and bacterial abundances on one hand, and between inhibiton diameters of antibiotic and abiotic parameters in the other hand. Statistical analyses were performed using SPSS software version 25.0. A p value < 0.05 was assumed to be significant.

3. Results
3.1. Physicochemical parameters
The physico-chemical parameters undergo spatiotemporal fluctuations. The figure 2 shows that the temperature varied between 19.2 and 31.5 °C with the lowest value recorded in stations Ws1 and Ws3 during the August campaign, while the highest value was recorded in station Ws3 during the February campaign, which corresponds to the dry season (Figure 2-A). The electrical conductivity fluctuated between 193 and 890 µS/cm. The lowest value was noted at station Ws2 during the March campaign, and the highest value at station Ws2 during the September campaign (Figure 2-B). The pH values varied between 6.5 and 8.21 C.U. The lowest value was recorded at stations Ws1 and Ws3 during the August campaign, and the highest value at station Ws1 during the September and October campaigns (Figure 2-C). TDS values fluctuated between 150 and 350 mg/L with the lowest value recorded at stations Ws3 and Ws1 during the November and June surveys respectively. The highest value was recorded in station Sw during the September campaign (Figure 2-D).

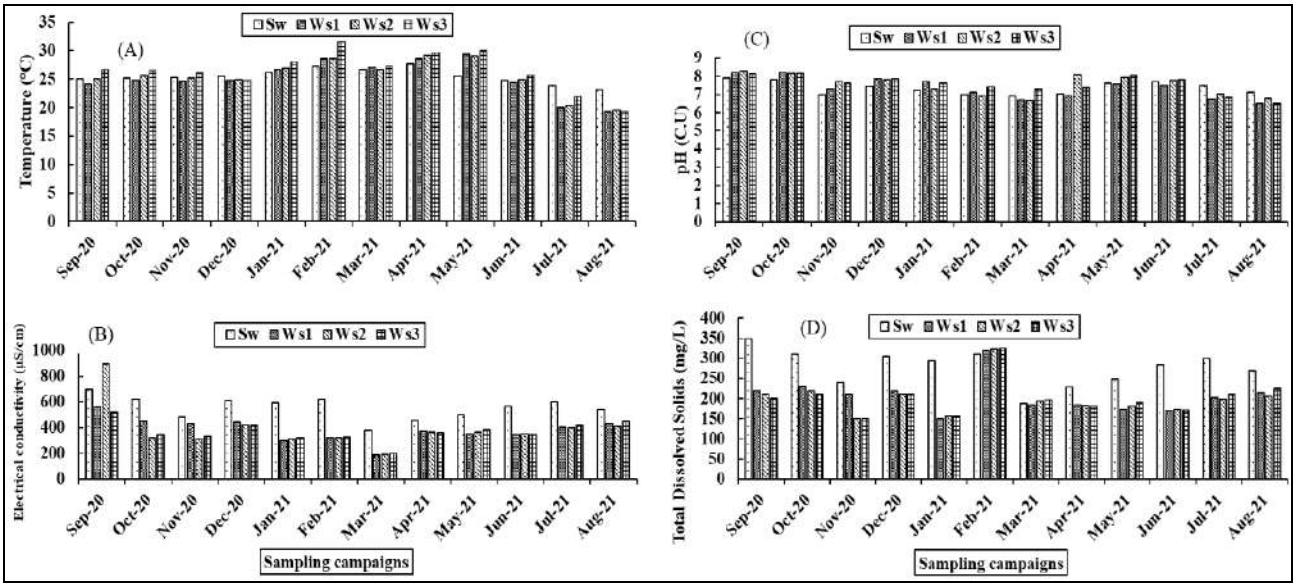
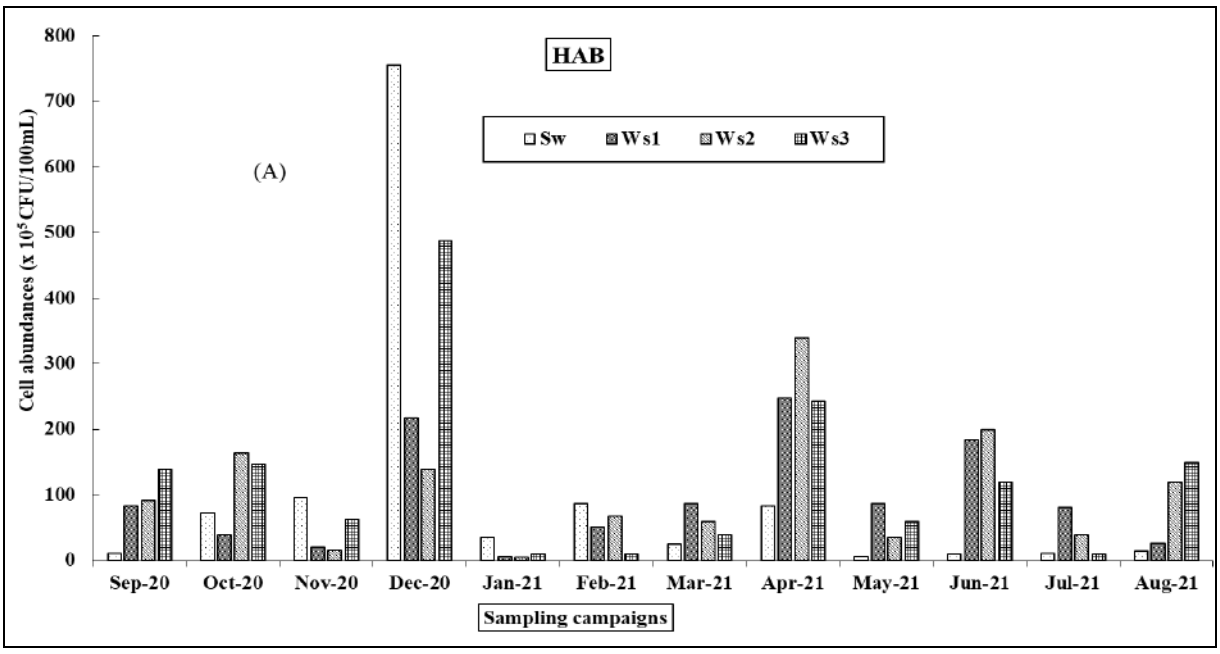


Fig 2: Variation of physicochemical parameters with respect to the different sampling sites and campaigns (A: variation of temperature; B: variation of electrical conductivity; C: variation of pH; D: variation of Total Dissolved Solid).

3.2. Bacteriological parameters

3.2.1. Bacterial abundance

The abundances of aerobic mesophilic heterotrophic bacteria (HAB) and *Proteeae* are shown in Figure 3. These abundances varied according to the campaigns and sampling station. In hospital wastewater, the abundance of HABs varied from 7 to 750 x 10^5 CFU/100mL with the lowest value recorded in the May survey, and the highest value in the December survey. While the abundance of *Proteeae* varied from 0 to 580 x 10^4 CFU/100mL with the lowest value recorded in the June survey and the highest in the December survey. On the other hand, in the water of the Mfoundi River, the abundance of HABs varied from 6 to 340 x 10^5 CFU/100mL, with the lowest and highest values recorded in station Ws2 during the January and April campaigns respectively. The abundance of *Proteeae* varied from 0 to 149 x 10^4 CFU/100mL. There were no bacteria recorded during the January campaigns in stations Ws1 and Ws2, and during the February and July campaigns in station Ws3; while the highest abundances was recorded during the December campaign in station Ws3 (Figure 3).



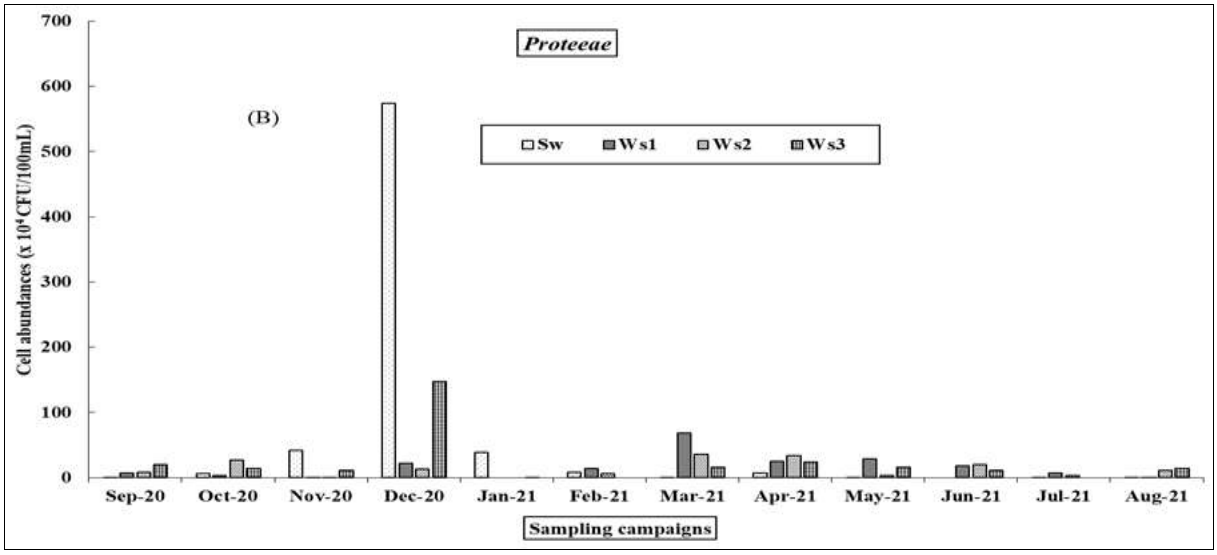


Fig 3: Variation of the abundances of total Heterotrophic Aerobe Bacteria (HAB) and *Proteeae* in the sampling sites

3.2.2. Diversity and abundance of *Proteus* species

Diversity of *Proteus* species

The *Proteus* species isolated from the different stations during the different sampling campaigns were *Proteus mirabilis* (*P. mirabils*), *Proteus penneri* (*P. penneri*) and *Proteus vulgaris* (*P. vulgaris*). This diversity varied according to the sampling site and the values of the Shannon and Weaver diversity index (H') showed that station Sw was the most diversed (H'= 1.009) and station Ws2 was the least diversed (H'= 0.802) (Table 2).

Table 2: Shannon and weaver diversity index

Sampling sites	Sw	Ws1	Ws2	Ws3
Index H'	1,009	0,847	0,802	0,988

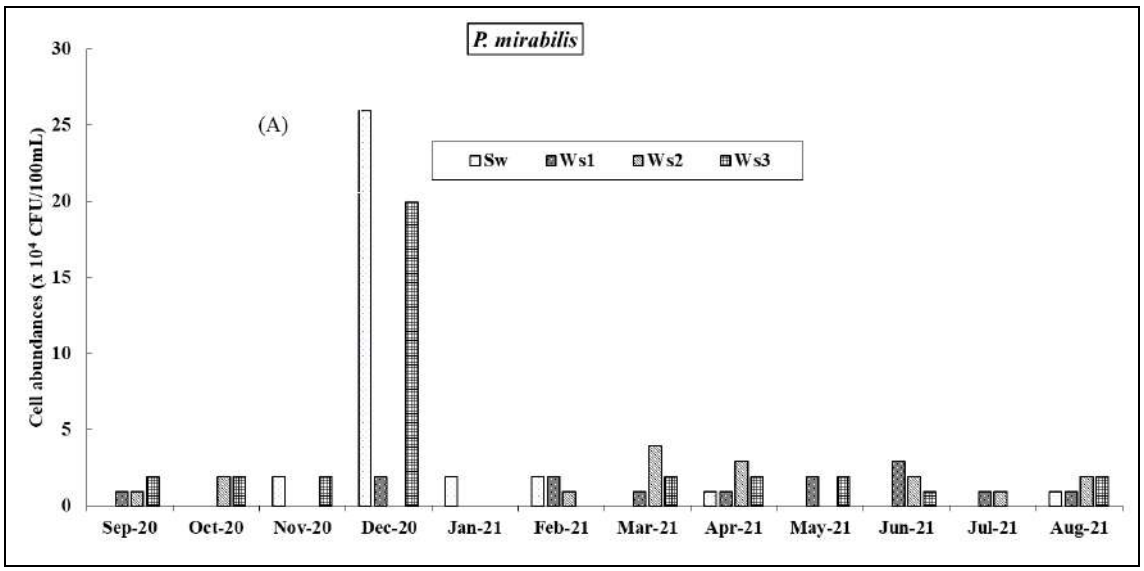
Abundance of *Proteus* species

The abundance of the *Proteus* species isolated varied with respect to the sampling station and campaign.

Abundance of *P. mirabilis* fluctuated between 0 and 26; 0 and 3; 0 and 4; 0 and 20 x 10^4 CFU/100mL at stations Sw, Ws1, Ws2 and Ws3 respectively. The lowest values were recorded in station Sw at the June campaign, in stations Ws1 and Ws2 at the January campaign, and in station Ws3 at the February and July campaigns. The highest values were recorded at the December survey in stations Sw and Ws3, and at the July and March surveys in stations Ws1 and Ws2 respectively (Figure 4-A).

Abundance of *P. penneri* ranged from 0 to 477; 0 to 48; 0 to 260; 0 to 35 x 10^4 CFU/100mL in stations Sw, Ws1, Ws2 and Ws3 respectively. The smallest values were recorded in June in station Sw, in January in stations Ws1 and Ws2 and during the February and July campaigns in station Ws3. The largest values were recorded in December in stations Sw and Ws3; and in March in stations Ws1 and Ws2 (Figure 4-B). *P. penneri* was the most represented species.

The isolated *P. vulgaris* species had abundance that varied between 0 and 73; 0 and 20; 0 and 20; 0 and 95 x 10^4 CFU/100mL in stations Sw, Ws1, Ws2 and Ws3 respectively. The smallest values were recorded in station Sw during the June campaign, in stations Ws1 and Ws2 during the January campaign, and in station Ws3 during the February and July campaigns. Large values were recorded at station Sw and Ws3 in the December survey; in March at station Ws1 and in October at station Ws2 (Figure 4-C).



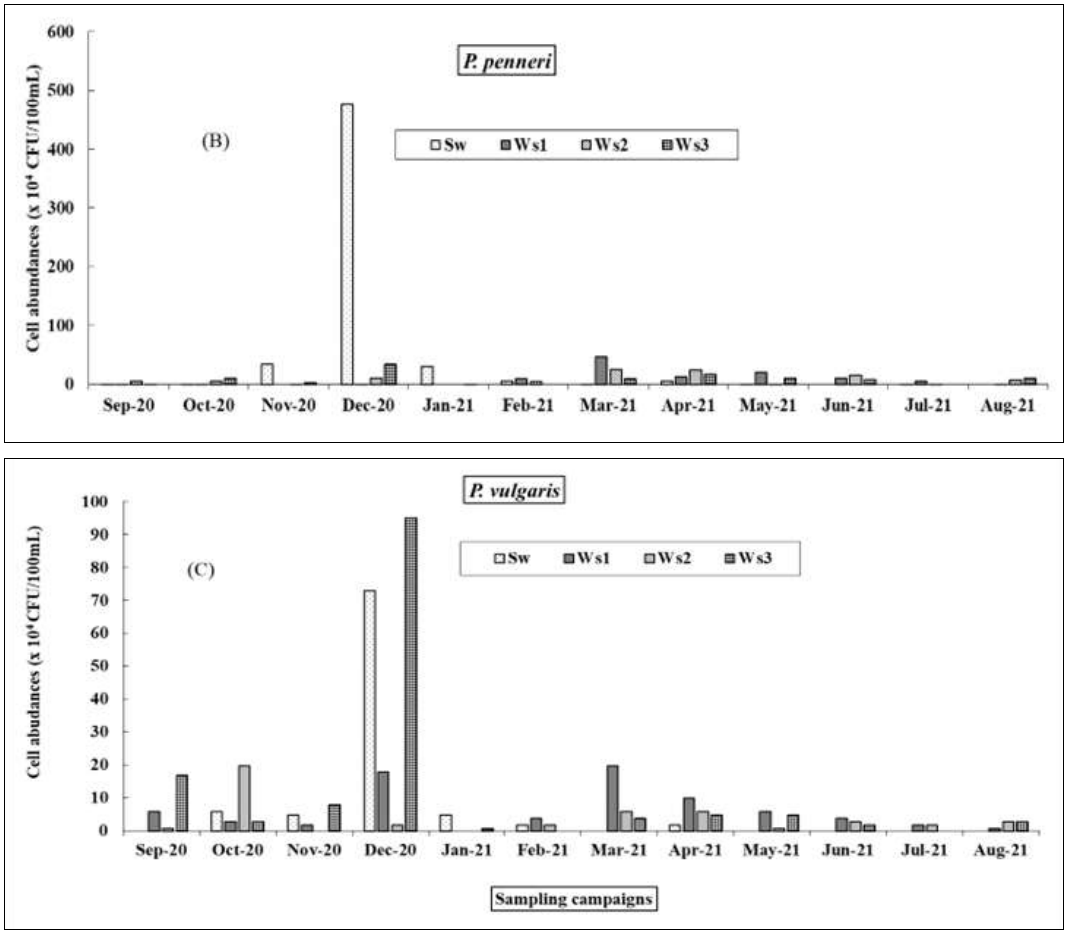


Fig 4: Abundance of *Proteus* species (*P. mirabilis*, *P. penneri*, *P. vulgaris*) isolated from different sampling points with respect to the sampling campaigns

3.2.3. Antibiotic susceptibility of *Proteus* species

3.2.3.1. Prevalence of resistance

The result of the antibiotic susceptibility testing showed that *Proteus* species isolated were resistant to almost all of the antibiotics tested. The prevalence of resistance represented in figure 5 varied according to the species and antibiotic families.

With the *Proteus* species isolated in Sw, it was observed that all *P. mirabilis* strains (100%) resisted to 10 β-lactam antibiotics (Amoxicillin, Ticarcillin, Piperacillin, Piperacillin+ Tazobactam, Ceftriaxon, Cefepim, Cefuroxim, Amoxicillin + Clavulanic acid, Imipenem and Ceftazidim) and they were slightly sensitive to 2 β-lactam antibiotics (Meropenem (25%) and Cefoxitin (15%). *P. penneri* strains were all resistant (100%) to the 12 β-lactams tested while *P. vulgaris* strains resisted to 11 β-lactam including Amoxicillin, Ticarcillin, Piperacillin, Piperacillin + Tazobactam, Ceftriaxon, Cefepim, Cefuroxim, Amoxicillin + Clavulanic acid, Imipenem, Cefoxitin and Ceftazidim. The resistance against Aminoglycoside family showed that there was high resistance rate against Gentamicin (90%, 100% and 75% respectively for *P. mirabilis*, *P. penneri* and *P. vulgaris* strains) and high sensitivity prevalence to Amikacin (100% for *P. mirabilis* strains and 50% for *P. penneri* and *P. vulgaris* strains). With the Quinolone family, the strains of the 3 *Proteus* species isolated were entirely resistant against Ciprofloxacin and Ofloxacin. However, there was a slight sensitivity of *P. mirabilis* and *P. vulgaris* strains to Norfloxacin (40%) and full resistance of *P. penneri* strains to this antibiotic.

In station Ws1, all *P. mirabilis*, *P. penneri* and *P. vulgaris* strains were resistant to the 3 antibiotic families considered (β-lactam, Aminoglysides and Quinolones).

Concerning the *Proteus* species isolated in station Ws2, it was noted that all *P. mirabilis* strains (100%) resisted to 10 β-lactam antibiotics (Amoxicillin, Meropenem, Ticarcillin, Piperacillin, Piperacillin + Tazobactam, Ceftriaxon, Cefepim, Cefuroxim, Cefoxitin and Ceftazidim) and highly sensitive to Imipenem (65%). *P. penneri* strains resisted to 11 β-lactam antibiotics including Amoxicillin, Meropenem, Ticarcillin, Piperacillin + Tazobactam, Ceftriaxon, Cefepim, Cefuroxim, Cefoxitin, Ceftazidim, Amoxicillin + Clavulanic acid and Imipenem. While *P. vulgaris* strains (100%) resisted to 9 β-lactam (Amoxicillin, Meropenem, Ticarcillin, Piperacillin, Piperacillin + Tazobactam, Ceftriaxon, Cefepim, Cefuroxim, and Ceftazidim); and slightly sensitive to Cefoxitin and Imipenem (25%). The resistance against Aminoglycoside family showed that *P. mirabilis* and *P. vulgaris* strains are highly susceptible to Gentamycin and Amikacin (75%). While, all *P. penneri* strains (100%) resisted to Gentamycin but to Amikacin, 60% of *P. penneri* strains were susceptible. With the Quinolone family, *P. mirabilis* and *P. vulgaris* strains (100%) resisted to Ciprofloxacin and Ofloxacin; but to Norfloxacin, 60% of strains of both species were susceptible. On the other hand, *P. penneri* strains are rather resisted (100%) to Norfloxacin and Ofloxacin.

In station Ws3, it was observed that all *P. mirabilis* and *P. vulgaris* strains (100%) were resistant to the 3 antibiotic families considered. However, the resistance against Quinolone family showed that 100% of *P. penneri* strains were resistant to 3 antibiotics tested; whereas face to

Aminoglycosides family, *P. penneri* strains are highly sensitive to Gentamycin (65%) and Amikacin (75%). With the β-lactam family, all *P. penneri* strains (100%) resisted to 9 β-lactam antibiotics (Amoxicillin, Meropenem, Ticarcillin, Piperacillin, Piperacillin + Tazobactam, Ceftriaxon, Cefuroxim, Cefoxitin and Ceftazidim), and they were slighly sensitive to 2 β-lactam antibiotics (Cefepim (20%) and Amoxicillin + Clavulanic acid (10%)).

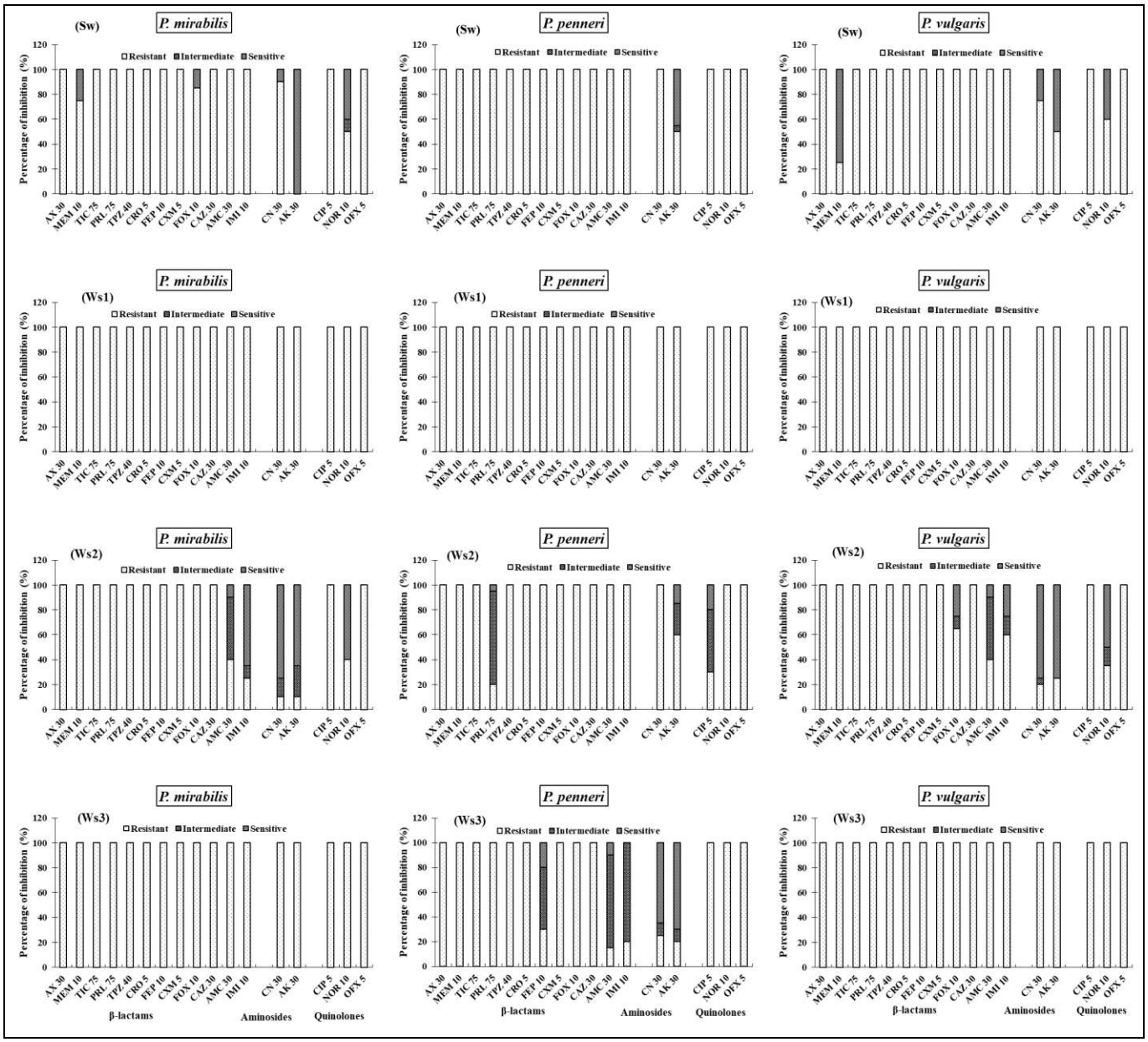


Fig 5: Prevalence of resistance of *Proteus* species against antibiotic tested AX: Amoxicillin; MEM: Meropenem; CIP: Ciprofloxacin; TIC: Ticarcillin; TPZ: Piperacillin + Tazobactam; CRO: Ceftriaxon; NOR: Norfloxacin; PRL: Piperacillin; FEP: Cefepim; CN: Gentamycin; FOX: Cefoxitin; OFX: Ofloxacin; AMC: Amoxicillin + Clavulanic acid; AK: Amikacin; CXM: Cefuroxim; CAZ: Ceftazidim; IMI: Imipenem.

3.2.4.3. Comparison tests

The Kruskal-Wallis and Mann-Whithney tests were performed to compare the antibiotic inhibition diameters of *Proteus* species isolated, between the different sampling sites taken two by two. The results are presented in Table 3.

A high significant difference (p<0.01) was noted between hospital wastewater and upstream river regarding the inhibition diameters of Meropenem, Cefoxitin, Imipenem, Gentamycin, Amikacin, Norfloxacin and Ofloxacin tested against *P. mirabilis* strains. The same observation was noted between hospital wastewater and landing of river; hospital wastewater and downstream river; upstream river and landing river; and between upstream and downstream river respectively for Meropenem, Imipenem, Amikacin and Norfloxacin; for Amikacin and Ceftriaxon; and for Cefuroxim, Cefoxitin and Norfloxacin. A significant difference (p<0.05) was observed between hospital wastewater and upstream river; upstream and landing river; and between landing and downstream river regarding respectively the inhibition diameters of Ceftriaxon and Cefuroxim; of Ceftriaxon, of Cefoxitin and Gentamycin; and finally for Norfloxacin against *P. mirabilis* strains isolated (Table 3).

In addition, a high significant difference (p<0.01) was noted between hospital wastewater and upstream river regarding the inhibition diameters of Cefuroxim, Ceftazidim, Imipenem, Amoxicillin + Clavulanic acid, Gentamycin, Amikacin, Norfloxacin and Ofloxacin tested against *P.*

penneri strains. The same observation was also made between hospital wastewater and landing river; hospital wastewater and downstream river; upstream and landing river; upstream and downstream river; and between landing and downstream river respectively for Meropenem and Ticarcillin; for Piperacillin, Amikacin and Norfloxacin; for Ticarcillin; for Piperacillin, Ceftriaxon and Amoxicillin + Clavulanic acid; and finally for Piperacillin. A significant difference ($p<0.05$) was also noted between hospital wastewater and upstream river; hospital wastewater and landing river; hospital wastewater and downstream river; upstream and landing river; upstream and downstream river; and between landing and downstream river regarding the inhibition diameters respectively of Meropenem and Ceftriaxon; of Cefuroxim and Norfloxacin, of Meropenem and Ticarcillin; of Ofloxacin; of Gentamycin; and finally of Ceftriaxon tested against *P. penneri* strains isolated (Table 3).

P. vulgaris strains showed a high significant difference ($p<0.01$) between hospital wastewater and upstream river regarding the inhibition diameters of Meropenem, Imipenem, Gentamycin, Amikacin and Norfloxacin. The same observation was noted between from hospital wastewater and landing river; hospital wastewater and downstream river; upstream and landing river; and between landing and downstream river respectively of Meropenem; of Meropenem, Piperacillin, Amoxicillin + Clavulanic acid, Amikacin and Norfloxacin; of Piperacillin, Ceftriaxon, Amoxicillin + Clavulanic acid and Gentamycin; and finally of Piperacillin. In addition, a significant difference ($p<0.05$) was also obtained between hospital wastewater and landing river; hospital wastewater and downstream river; upstream and landing river; and between upstream and downstream river regarding the inhibition diameters respectively of Amikacin, Norfloxacin and Ofloxacin; of Ceftriaxon and Ofloxacin; of Amikacin and Ofloxacin; and finally of Imipenem and Ofloxacin tested against *P. vulgaris* strains (Table 3).

Table 3: P-values of the Mann-Whitney test comparing the antibiotic inhibition diameters between the different sampling sites taken 2 by two

Antibiotics (mcg)	Types of water taken 2 by two and bacterial species considered					
	Hospital wastewater and Upstream river	**Hospital wastewater and Landing river**	**Hospital wastewater and Downstream river**	**Upstream and Landing river**	**Upstream and Downstream river**	**Landing and Downstream river**
P. mirabilis						
MEM (10)	0.000**	0.000**	0.000**	0.577	0.195	0.538
CRO (5)	0.012*	0.884	0.541	0.018*	0.001**	0.398
CXM (5)	0.020*	0.282	0.332	0.748	0.001**	0.096
FOX (10)	0.005**	0.561	0.953	0.034*	0.003**	0.415
IMI (10)	0.005**	0.642	0.004**	0.210	0.641	0.180
CN (30)	0.008**	1.000	0.221	0.024*	0.080	0.431
AK (30)	0.000**	0.416	0.002**	0.005**	0.317	0.024*
NOR (10)	0.001**	0.435	0.000**	0.885	0.001**	0.051
OFX (5)	0.003**	0.560	0.081	0.052	0.116	0.400
P. penneri						
MEM (10)	0.015*	0.008**	0.026*	0.770	0.210	0.116
TIC (75)	0.088	0.001**	0.038*	0.009**	0.519	0.085
PRL (75)	0.165	0.464	0.002**	0.794	0.000**	0.006**
CRO (5)	0.018*	0.199	0.839	0.245	0.001**	0.046*
CXM (5)	0.002**	0.027*	0.109	0.559	0.139	0.116
CAZ (30)	0.001**	0.156	0.082	0.523	0.172	0.750
IMI (10)	0.001**	0.059	0.173	0.907	0.431	0.861
AMC (30)	0.006**	0,257	0.816	0.954	0.004**	0.212
CN (30)	0.008**	0.366	0.433	0.184	0.011*	0.244
AK (30)	0.000**	0.118	0.001**	0.145	0.520	0.146
NOR (10)	0.003**	0.013*	0.001**	0.153	0.116	0.839
OFX (5)	0.002**	0.661	0.233	0.010*	0.077	0.171
P. vulgaris						
MEM (10)	0.000**	0.000**	0.000**	0.320	0.396	0.768
PRL (75)	0.408	0.724	0.000**	0.334	0.001**	0.001**
CRO (5)	0.335	0.771	0.037*	0.194	0.000**	0.061
IMI (10)	0.009**	0.265	0.522	0.105	0.039*	0.790
AMC (30)	0.447	0.580	0.005**	0.266	0.000**	0.098
CN (30)	0.001**	0.054	0.399	0.107	0.004**	0169
AK (30)	0.000**	0.036*	0.003**	0.010*	0.121	0.218
NOR (10)	0.000**	0.010*	0.001**	0.884	0.749	0.839
OFX (5)	0.400	0.015*	0.045*	0.011*	0.039*	0.434

*: $p<0.05$; ** $p<0.01$; MEM: Meropenem; TIC: Ticarcillin; CRO: Ceftriaxon; NOR: Norfloxacin; PRL: Piperacillin; CN: Gentamycin; FOX: Cefoxitin; OFX: Ofloxacin; AMC: Amoxicillin + Clavulanic acid; AK: Amikacin; CXM: Cefuroxim; CAZ: Ceftazidim; IMI: Imipenem.

3.2.4.4. Correlations amongst the considered parameters

The Spearman correlation test showed an increase in temperature that led to a significant increase of the abundance of *P. mirabilis* ($p<0.05$, r = 0.58) and *P. penneri* ($p<0.05$, r = 0.62) isolated from hospital wastewater (Table 4).

Table 4: Correlation coefficients between physicochemical and bacteriological parameters

Hospital wastewater (Sw)				Upstream river (Ws1)			Landing river (Ws2)			Downstream river (Ws3)		
Abiotic parameters	**Bacteriological parameters**											
	P. mi	***P. pen***	***P.vul***	***P. mi***	***P. pen***	***P.vul***	***P. mi***	***P. pen***	***P.vul***	***P. mi***	***P. pen***	***P.vul***
Temp	**0.58***	**0.62***	0.45	0.35	0.46	0.47	0.00	-0.03	-0.06	-0.25	-0.09	-0.04
pH	-0.39	-0.48	-0.15	-0.12	-0.45	0.08	-0.11	-0.01	0.04	0.13	0.18	0.43
E. cond	0.13	0.03	0.24	-0.30	-0.48	-0.04	0.02	0.19	0.00	0.14	0.26	0.31
TDS	0.13	0.03	0.24	-0.08	-0.25	0.08	0.34	0.23	0.39	0.08	-0.08	-0.29

*: $p<0.05$; *P.mi: Proteus mirabilis; P. pen: Proteus penneri; P. vul: Proteus vulgaris*; Temp: temperature; E. cond: electrical conductivity; TDS: total dissolved solids.

The Spearman correlation test was also carried out between the inhibition diameters of antibiotics and abiotics parameters of hospital wastewater and river. The results showed that in hospital wastewater, there is a positive and significant relationship ($p<0.05$) between the *P. mirabilis* susceptibility to Ceftazidim and temperature (r = 0.65); Piperacillin + Tazobactam, Cefoxitin, Ofloxacin and electrical conductivity and TDS (r = 0.15; r = 0.61; r = 0.62 respectively). A negative and significant relationship ($p<0.05$; r = -0.68) is also observed between *P. mirabilis* susceptibility against Ceftazidim and pH (Table 5). On the contrary, a negative and high significant relationship ($p<0.01$) was observed between the susceptibility of the same species to Ofloxacin and temperature (r = -0.7); Ceftriaxon and pH (r = -0.7); Ciprofloxacin and electrical conductivity and TDS (r = -0.7). Concerning the relationship between the *P. penneri* susceptibility against antibiotics and water abiotics factors, a positive and high significant relationship ($p<0.01$) was noted between the susceptibility of this bacteria to Piperacillin + Tazobactam, Ceftriaxon, Amoxicillin + Clavulanic acid and temperature (r = 0.7; r = 0.8; r = 0.7 respectively); and a negative and high significant relationship ($p<0.01$) between the susceptibility of the same bacteria to Ceftriaxon and pH (r = -0.7). In contrast, a positive and significant relationship ($p<0.05$) was noted between the susceptibility against Ceftazidim and temperature (r = 0.59). However, for *P. vulgaris*, there is a positive and significant relationship ($p<0.05$) between the susceptibility of this species to only Piperacillin and electrical conductivity and TDS (r = 0.58) (Table 5).

Table 5: Correlation coefficients between inhibition diameters of each antibiotic and physicochemicals parameters of hospital wastewater

Antibiotics	**Abiotics parameters of hospital wastewater (A)**											
	Temp	**pH**	**E.cond**	**TDS**	**Temp**	**pH**	**E.cond**	**TDS**	**Temp**	**pH**	**E.cond**	**TDS**
	P. mirabilis				***P. penneri***				***P. vulgaris***			
AX	0.397	-0.28	-0.128	-0.13	0.025	-0.53	0.141	0.141	-0.29	0.351	0.493	0.493
MEM	0.296	-0.03	0.321	0.321	0.308	0.149	0.088	0.088	-0.37	0.224	0.540	0.540
TIC	-0.043	-0.210	-0.132	-0.13	-0.15	-0.02	-0.05	-0.05	0.339	-0.55	-0.47	-0.47
PRL	-0.187	-0.224	0.224	0.224	-0.35	0.260	0.119	0.119	-0.05	0.347	0.58*	0.58*
TPZ	0.195	-0.03	0.15*	0.15*	0.7**	-0.31	-0.18	-0.18	-0.42	0.203	-0.266	-0.26
CRO	0.222	-0.7**	-0.121	-0.12	0.8**	-0.7**	-0.487	-0.47	0.041	-0.27	0.169	0.169
FEP	0.246	-0.15	0.459	0.459	-0.17	-0.04	0.474	0.474	-0.45	0.199	0.285	0.285
CXM	0.526	-0.266	0.088	0.088	-0.01	0.419	-0.03	-0.03	-0.03	-0.92	-0.46	-0.46
FOX	-0.568	0.425	0.61*	0.61*	0.005	-0.13	0.239	0.239	0.265	0.167	0.394	0.394
CAZ	0.65*	-0.68*	-0.402	-0.40	0.59*	-0.52	-0.127	-0.12	0.197	-0.31	0.179	0.179
IMI	0.498	-0.218	0.018	0.018	0.564	-0.37	-0.021	-0.02	0.524	-0.25	0.088	0.088
AMC	-0.529	0.102	0.694	0.694	0.7**	-0.09	0.055	0.055	0.178	0.146	-0.335	-0.33
CN	-0.165	0.026	0.488	0.488	0.072	-0.35	-0.85	-0.85	0.248	-0.23	0.341	0.341
AK	-0.014	0.032	0.228	0.288	-0.04	-0.20	0.046	0.046	0.046	-0.19	-0.109	-0.11
CIP	0.283	-0.366	-0.7**	-0.7**	-0.16	-0.13	-0.481	-0.48	-0.45	0.387	-0.236	-0.24
NOR	0.258	0.058	0.232	0.232	0.165	-0.28	0.182	0.182	0.502	-0.89	-0.058	-0.06
OFX	-0.7**	0.542	0.62*	0.62*	-0.39	0.466	0.485	0.485	-0.38	0.460	-0.037	-0.04

*****: $p<0.05$; ****** $p<0.01$; Temp: temperature; E. cond: electrical conductivity; TDS: total dissolved solids; AX: Amoxicillin; MEM: Meropenem; TIC: Ticarcillin; PRL: Piperacillin; TPZ: Piperacillin + Tazobactam; CRO: Ceftriaxon; FEP: Cefepim; CXM: Cefuroxim; FOX: Cefoxitin; CAZ: Ceftazidim; IMI: Imipenem; AMC: Amoxicillin + Clavulanic acid; CN: Gentamycin; AK: Amikacin; CIP: Ciprofloxacin; NOR: Norfloxacin; OFX: Ofloxacin.

Considering the correlation coefficients between inhibition diameters of each antibiotic and physicochemical parameters of river, the results showed that there is a positive and high significant relationship ($p<0.01$) between the *P. mirabilis* susceptibility to Cefuroxim and electrical conductivity (r = 0.74), and a negative and high significant relationship ($p<0.01$) between the susceptibility to Ceftriaxon, Cefuroxim and temperature (r= -0.72 and r= -0.78) on one hand; and Piperacillin + Tazobactam and electrical conductivity (r = -0.7) on the other hand. A positive and significant relationship ($p<0.05$) between the susceptibility to Ceftriaxon and electrical conductivity (r= 0.64); and a negative and significant relationship ($p<0.05$) between the susceptibility to Ofloxacin and temperature (r = -0.60) is noted (Table 6). Concerning *P. penneri*, there is a positive and high significant relationship ($p<0.01$) between the susceptibility of this specie to Ceftriaxon, Cefuroxim and electrical conductivity (r = 0.72 and r = 0.88); and a negative and high significant relationship ($p<0.01$) between the same bacteria susceptibility to Cefuroxim and

temperature (r = -0.7). However, a positive and significant relationship (p<0.05) between the *P. penneri* susceptibility to Ofloxacin and electrical conductivity (r = 0.68), and a negative and significant relationship (p<0.05) between the susceptibility of the same bacteria to Amoxicillin, Ceftriaxon, Ofloxacin and temperature (r= -0.6; r = -0.7 and r = -0.7 respectively) on one hand; and Piperacillin + Tazobactam and electrical conductivity (r = -0.59) on the other hand are noted. About *P. vulgaris*, a positive and high significant relationship (p<0.01) was obtained between their susceptibility to Cefuroxim and electrical conductivity (r = 0.7). However, there is a positive and significant relationship (p<0.05) between the *P. vulgaris* susceptibility to Meropenem and pH (r = 0.59) on one hand, and Ceftriaxon, Oflaxacin and electrical conductivity (r = 0.62 and r = 0.68) on the other hand. A negative and significant relationship (p<0.05) was noted between the susceptibility of the same bacteria to Ceftriaxon, Cefuroxim, Ofloxacin and temperature on one hand (r= -0.6; r = -0.7 and r = -0.6 respectively); and Ticarcillin and electrical conductivity (r = -0.57) on the other hand (Table 6).

Table 6: Correlation coefficients between inhibition diameters of each antibiotic and physicochemical parameters of surface water

Antibiotics	Abiotics parameters of surface water (B)											
	Temp	**pH**	**E.cond**	**TDS**	**Temp**	**pH**	**E.cond**	**TDS**	**Temp**	**pH**	**E.cond**	**TDS**
	P. mirabilis				***P. penneri***				***P. vulgaris***			
AX	-0.481	0.384	0.283	0.159	**-0.6***	0.387	0.346	0.134	-0.38	0.457	0.280	0.037
MEM	-0.319	0.453	0.309	0.232	-0.42	0.495	0.465	0.255	-0.25	0.59*	0.250	0.014
TIC	0.030	-0.214	-0.246	0.229	0.223	-0.02	0.067	0.050	0.499	-0.02	-0.57*	-0.29
PRL	0.336	0.139	-0.294	-0.05	-0.34	0.165	0.379	0.333	0.067	0.402	0.113	0.051
TPZ	0.503	-0.113	-0.7**	0.016	0.445	-0.09	-0.59*	-0.02	0.470	0.197	-0.416	-0.06
CRO	-0.72**	0.057	0.604*	0.409	-0.7*	0.124	0.72**	0.234	-0.6*	0.114	0.62*	0.21
FEP	-0.322	0.481	0.405	0.242	-0.20	0.477	0.356	0.189	-0.15	0.473	0.306	0.137
CXM	-0.78**	0.042	0.74**	0.239	-0.7**	0.067	0.8**	0.055	-0.7*	-0.05	0.7**	0.12
FOX	-0.346	0.321	0.212	0.194	-0.36	0.511	0.366	0.114	-0.36	0.491	0.343	0.111
CAZ	-0.366	0.368	0.212	0.108	-0.38	0.390	0.238	0.086	0.075	0.431	-0.131	-0.22
IMI	-0.529	0.320	0.478	0.355	-0.15	0.404	0.165	0.047	-0.32	0.558	0.398	0.150
AMC	-0.364	0.469	0.366	0.201	-0.37	0.406	0.308	0.200	0.408	0.488	0.016	0.113
CN	-0.358	0.359	0.470	0.479	-0.12	0.218	0.08	0.229	0.073	0.341	0.084	0.138
AK	-0.216	0.2337	0.278	0.427	-0.08	0.395	0.304	0.292	-0.24	0.159	0.270	0.232
CIP	0.324	0.251	-0.246	0.034	-0.28	0.116	0.258	0.253	-0.17	0.430	0.180	-0.05
NOR	-0.224	0.292	0.183	0.194	-0.12	0.512	0.267	0.069	-0.67	0.327	0.608	0.242
OFX	-0.674*	0.134	0.490	0.355	-0.7*	0.240	0.68*	0.337	-0.6*	0.460	0.68*	0.04

*****: p<0.05; ****** p<0.01; Temp: temperature; E. cond: electrical conductivity; TDS: total dissolved solids; AX: Amoxicillin; MEM: Meropenem; TIC: Ticarcillin; PRL: Piperacillin; TPZ: Piperacillin + Tazobactam; CRO: Ceftriaxon; FEP: Cefepim; CXM: Cefuroxim; FOX: Cefoxitin; CAZ: Ceftazidim; IMI: Imipenem; AMC: Amoxicillin + Clavulanic acid; CN: Gentamycin; AK: Amikacin; CIP: Ciprofloxacin; NOR: Norfloxacin; OFX: Ofloxacin.

4. Discussion

The results of the physicochemical parameters obtained showed that the values fluctuated depending on the stations and sampling periods. Regarding temperature, it was observed that this fluctuation was much more pronounced in surface waters (19.2 - 31.5 °C) than in hospital wastewater (21 - 26.2 °C) and higher during the surveys carried out in the dry season. This could be explained by the variation of the ambient temperature of the environment and the time of sunshine as high values were obtained in the dry season and low values in the rainy season. These results are similar to those obtained by Tuekam [30] in the waters of the Mfoundi catchment. According to Merhabi *et al*. [31], surface water temperature is affected by fluctuating rainfall and seasonal temperatures. Temperature is an important abiotic factor as it governs almost all physicochemical and biological reactions.

The average values of electrical conductivity (700±1.09 µS/Cm) and total dissolved solids (200±1.06 mg/L) of the Mfoundi River showed that they are more mineralized and more charged with dissolved matter contrary to hospital wastewater whose average values of electrical conductivity and TDS were respectively (560±1.90 µS/Cm) and (140±1.79 mg/L). This could be justified by the fact that the Mfoundi receives water from neighbouring tributaries and waste from anthropogenic activities loaded with ion-rich dissolved matter as these two physicochemical parameters describe the presence of inorganic salts in solution [32]. These results of high mineralization of the Mfoundi River are far from those obtained by Tuekam [30] who showed that rivers have low mineralization because they are less anthropized. According to Ajeagah *et al*. [33], the high values of electrical conductivity and total dissolved solids can be explained by the high degradation of organic matter present in the environment and could reflect the high pollution of surface waters.

The average pH value of surface water was (8.01±1.01 C.U) showing that this water is alkaline unlike that of hospital wastewater (6.13±1.98 C.U) which is slightly acidic. This difference may be due to the nature of each type of water, which would justify that surface water is alkaline because this water is by nature loaded with organic matter, which increases its basicity. These basic pH values obtained in the Mfoundi River are similar to those recorded by Noah *et al*. [34] in the Mefomo River in the Central Cameroon region. Indeed, the pH of water depends on its origin, the nature of the soil it flows through, the presence of microorganisms and the anthropic activities carried out there [21, 35].

The Shannon and Weaver diversity index showed that hospital wastewater has a more diversified microbiota (H'= 1.009) than surface water (H'=0.879). This could be explained by the fact that these waters receive many bacterial germs from patients. According to Olalemi *et al*. [36], hospital wastewater harbours various species of *Proteus*

because they are very abundant clinical germs in hospitals. Of the 3 *Proteus* species isolated in this study, *P. penneri* species is the most abundant in both hospital wastewater and surface water. Contrary to several works, *P. mirabilis* and *P. vulgaris* species are more abundant than *P. penneri* species especially in hospital waters [37, 38, 39]. The high prevalence of *P. penneri* could be due to the influence of abiotic factors on this species.

The relationship between bacterial abundances and physicochemical parameters shows that the increase in temperature is significantly correlated with the increase in abundances of *P. mirabilis* and *P. penneri* isolated from hospital wastewater; this would explain the high abundance of these 2 species recorded in this study. Studies conducted by Mohamed *et al*. [40] showed that bacterial loads correlate with environmental parameters measured periodically at any point of the site; and according to Merhabi *et al*. [30], temperature has a very significant influence on the abundance of *Enterobacteriaceae* and consequently that of *Proteus*.

The antibiotic susceptibility test shows that the *Proteus* species isolated from the different sampling sites were highly resistant to several antibiotics that were used. This multi-resistance was observed in the different families of antibiotics tested and this corroborate the work of Pierre [41] who showed that all *Enterobacteriaceae* species including genus *Proteus* expressed multi-resistant to antibiotics. According to Bonnet [42], this multi-resistance results from four mechanisms: impermeability, efflux, modification of the target of the antibiotic (PLP) and enzyme production.

Isolated *Proteus* species expressed a high resistance to β-lactams (95.10%) and Quinolones (81.09%). According to Bonnet [42], most *Proteus* species are naturally resistant to β-lactams due to the various β-lactamases they secrete; but on the other hand naturally sensitive to Quinolones. The resistance observed in *Proteus* species to Quinolones could be due to the acquired resistance mechanisms favoured by environmental factors. These results corroborate with those of Djombera [43] which reveal the high resistance of *Proteus* species to Quinolones. However, other previous work has shown that resistance to β-lactams depends on each type of *Proteus* species. *P. mirabilis* due to its wild phenotype is naturally susceptible to β-lactams unlike other *Proteus* species [44]; this does not corroborate with the results obtained and leads to the conclusion that the isolated *P. mirabilis* species are not wild strains.

With the Aminoglycoside's family, it was noted that *Proteus* species had a slight resistance to Gentamicin (41.8%) and a high sensitivity to Amikacin (87.5%). The approximate results were obtained by Djombera [43] showing that 47.83% of *Proteus* species resisted to Gentamicin and 68.6% were sensitive to Amikacin. According to Rajiv *et al*. [37], all *Proteus* species are highly susceptible to Gentamicin and Amikacin. However, resistance to Gentamicin is thought to be due to enzymatic inactivation which is the most common mechanism of acquired resistance in *Proteus* [42].

Although there was a high sensitivity to Gentamicin and Amikacin with *P. mirabilis* and *P. vulgaris* species isolated from the middle stream of Mfoundi, this was not the case when they were isolated from hospital wastewater as they were resistant to the 2 Aminoglycosides tested. Similar results were obtained by Chen *et al*. [45] showing a high resistance (80%) of *Proteus* species to Gentamicin and Amikacin. This would mean that the environmental conditions leading in sampling site where *Proteus* species were isolated influence their susceptibility to antibiotics by facilitating the acquisition of resistance mechanisms. According to Amara [46], the antibiotic resistance of *Proteus* species is greater when isolated in hospital environments due to the various antibiotics residues present in these waters which lead to an increased level of resistance. The wide use of Aminoglycosides contributes to the emergence of resistant strains [47].

The correlation test performed showed significant relationship between antibiotic inhibition diameter measured in *P. mirabilis* strains and physicochemical parameters such as temperature, electrical conductivity, pH and Total Dissolved Solids. These results are similar to those of Signe *et al*. [48] who noted some significant correlations between some physicochemical water parameters and antibiotic inhibition diameters. This demonstrates that the susceptibility of some bacterial species may be regulated by a complex mechanism including some abiotic characteristics of the water [49, 50]. The lack of correlation between physicochemical parameters and Aminoglycosides (Gentamicin and Amikacin) justifies the sensitivity of *Proteus* to both antibiotics. These results are similar to those of Bentroki *et al*. [51] confirming the sensitivity of *Proteus* to Gentamicin and Amikacin.

The Mann-whitney comparison test showed that there was a significant difference of antibiotic inhibition diameters measured in *Proteus* species between hospital wastewater and Mfoundi River. This could be explained by the different features of the two types of water which can contain immense genetic variability, opportunities for mutation, rearrangement and horizontal gene transfer. Indeed, new resistance genes could relatively be due to a strong pressure to maintain them [52, 53]. In addition, significant differences of antibiotic inhibition diameters were also noted between upstream and downstream of the Mfoundi River. This could be justified by the diversity of the quality of water they receive as well as the anthropogenic activities that take place near the upstream and downstream of the river. The susceptibility of bacteria to antibiotics can be impacted by several environmental factors [49, 50].

5. Conclusion

Hospital wastewater and Mfoundi River harbour several *Proteus* species such as *P. mirabilis*, *P. penneri* and *P. vulgaris*. They are more represented in hospital wastewater, highly resistant to β-lactams and Quinolones and slightly sensitive to Aminoglycosides. The high prevalence of antibiotic resistance noted with *P. mirabilis* strains could be linked to environmental factors that can modify the wild phenotype of this species or due to the acquisition of resistant genes. The multi-resistance of *Proteus* species would be due to the multiple use of antibiotics both in hospitals and in the community. This represents a health risk for humans and the aquatic environment.

6. Compliance with ethical standards

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7. Disclosure of conflict of interest

None conflict of interest to declare. The manuscript has not been previously submitted or published in other journal and is not being considered for publication elsewhere.

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